

Towards a Maraviroc Long-Acting Injectable Nanoformulation

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Abstract

Suboptimal adherence to antiretroviral (ARV) therapy can lead to insufficient drug exposure leading to viral rebound and increased likelihood of resistance. This has driven the development of long-acting injectable (LAI) formulations which may mitigate some of these problems. Maraviroc (MVC) is an orally dosed CCR5 antagonist approved for use in patients infected with CCR5-trophic HIV-1. MVC prevents viral entry into host cells, is readily distributed to biologically relevant tissues and has an alternative resistance profile compared to more commonly used therapies. This makes a MVC LAI formulation particularly appealing for implementation in Pre-Exposure Prophylaxis (PrEP). A 70 wt.% MVC-loaded nanodispersion stabilised with polyvinyl alcohol (PVA) and sodium 1,4-bis(2-ethylhexoxy)-1,4-dioxobutane-2-sulfonate (AOT) was prepared using emulsion-templated freeze-drying. *In vitro* release rate studies revealed over a 22% decrease in MVC release rate constant across a size selective membrane compared with an aqueous solution of MVC (<5% DMSO). Pharmacokinetic studies in rats were subsequently carried out following intramuscular injection of either the nanodispersion or an aqueous MVC preparation (<5% DMSO). Results demonstrated over a 3.4-fold increase in AUC_{0-∞} (1959.71 vs 567.17 ng.h ml), over a 2.6-fold increase in MVCs terminal half-life (t_{1/2}) (140.69 vs 53.23 h) and MVC concentrations present up to 10-days. These data support development of a MVC LAI formulation with potential application in HIV therapy or prevention.

Keywords

Maraviroc; Long-Acting Injectable (LAI); Long-Acting Parenteral (LAP); Intramuscular; Nanodispersion; Nanomedicine; Pharmacokinetics; Pre-exposure Prophylaxis (PrEP)

Introduction

The introduction of antiretroviral therapy (ART) has significantly reduced HIV-associated morbidity and mortality and has transformed HIV infection into a manageable chronic condition. Currently, there are over 20 antiretrovirals (ARVs) from 6 drug classes and multiple effective first-line regimens for HIV-1 treatment [1]. Despite these advances, strict adherence to daily oral ART remains essential in maintaining viral suppression, preventing the emergence of resistance to therapy and reducing the risk of HIV transmission [2,3]. Additionally, insufficient drug concentrations at anatomically important locations has been shown to lead to persistent viral replication and maintenance of the disease [4,5]. Pre-exposure prophylaxis (PrEP) using ART has been shown to be effective in the prevention of HIV acquisition in individuals identified as being at risk of infection [6]. Currently, the only drugs used for HIV-1 PrEP are once-daily orally administered tenofovir, tenofovir/emtricitabine or tenofovir/lamivudine [7,8]. Studies have shown a clear dose-response relationship between protection and adherence to therapy [9]. The challenges presented by daily oral dosing and the requirement for life-long maintenance of such dosing has driven interest in the development of more convenient dosing schedules for both HIV treatment and PrEP.

A number of strategies have been used to deliver long-acting therapeutics including implants and injectables. Long-acting reversible contraception methods such as the levonorgestrel subdermal hormone implant provides a reversible and highly effective means of long-term pregnancy prevention. The implant consists of two sealed silastic tubes, each containing 75 mg levonorgestrel which provides up to 5 years of effective contraceptive protection [10,11]. Subdermal implants have, until recently, received little attention for the delivery of ARVs. However, implants containing the prodrug tenofovir alafenamide (TAF) are currently being developed towards PrEP applications. A novel subdermal TAF implant, consisting of a TAF core inside a silicone scaffold was pharmacologically assessed in beagle dogs. The implant was shown to maintain a low systemic plasma exposure of both TAF and tenofovir (TFV) for 40 days. High concentrations of the pharmacologically active metabolite, TFV diphosphate (TFV-DP), was observed in peripheral blood mononuclear cells (PBMCs) at levels over 30-fold greater than required for HIV PrEP in humans [12]. More recently, a biodegradable TAF containing subcutaneous implant for HIV PrEP was assessed in New Zealand White rabbits. The pharmacokinetic data revealed that plasma TAF concentrations were detectable up to 70 days following implantation and that plasma TFV and PBMC TFV-DP concentrations were sustained throughout the 3-month study. Additionally, TFV-DP was detectable in vaginal, cervical and rectal tissues at 49 days, but had declined by day 91 [13].

Another strategy that is attracting interest is the development of long-acting injectables (LAIs), the concepts for which were initially developed for antipsychotic therapies [14,15] and contraception [16]. Currently, two solid drug nanoparticle (SDN) ARVs; rilpivirine and cabotegravir have entered clinical development as LAI formulations both with HIV treatment and prevention potential [17,18]. This potential was demonstrated in the phase 2b clinical trial; LATTE-2, involving treatment-naïve HIV-1 infected patients. In the trial, a once daily, three-drug, orally dosed ART (cabotegravir 30 mg; abacavir-lamivudine 600 mg – 300 mg) was compared to a long-acting intramuscular dose of cabotegravir plus rilpivirine at either a 4-week (400 mg; 600 mg, respectively) or 8-week dosing interval (600 mg; 900 mg, respectively). Results from the trial indicated that the long-acting injectable 4-week and 8-week regimens were well accepted and tolerated by patients and maintained virological suppression at rates comparable to a daily oral three-drug regimen [19]. Recently, a dolutegravir (DTG) prodrug preparation was created and encapsulated into poloxamer solid drug nanocrystals to produce a long-acting parenteral formulation. Pharmacokinetic analysis of DTG nanoparticles and the DTG-prodrug nanoparticles was carried out over 8 weeks following intramuscular injection in mice. DTG half-life was increased from 61.9 h to 330.4 h for the prodrug-loaded nanocrystals and average blood DTG concentrations remained above the PA-IC₉₀ for 8-weeks and tissue concentrations remained above the PA-IC₉₀ for 4-weeks. It was noted that drug nanocrystals were observed inside tissue macrophages and stored in the endosomes and autophagosomes. It is suggested that a secondary depot within the tissue macrophages, independent of the muscle at the site of injection, developed and influenced DTG exposure [20]. In addition to providing extended drug exposure, mitigating the need for daily oral dosing of potentially poorly bioavailable ARVs, LAI preparations have the potential for reducing drug metabolism, reducing gastrointestinal toxicity, and avoiding some drug-drug interactions [21]. The mechanisms which underpin drug release from this route of administration are currently not well understood, but data are beginning to emerge [22–25].

Maraviroc (MVC) has particular appeal for implementation in PrEP. It is readily absorbed into cervicovaginal and rectal tissues and is detectable in seminal plasma [26,27]. Recent studies have highlighted concerns regarding the emergence of drug-resistant HIV strains in patients who become infected with HIV whilst receiving PrEP [28,29]. MVC is a CCR5 antagonist and has a unique resistance profile compared to other ARVs. It is indicated for use in combination with other ARVs for the treatment of only CCR5-tropic HIV-1 infection in patients 2 years of age and older weighing ≥10 kg but is not commonly used in front-line therapy, even though resistance is rare [30,31]. Given MVCs unique resistance profile, it is unlikely that resistance will develop towards other mainstream front-line future therapy options should a patient become infected with HIV whilst receiving MVC PrEP. In addition, HIV-1 infection usually

occurs through infection with CCR5-tropic virus meaning MVC may be particularly useful in PrEP.

The efficacy of orally dosed MVC containing PrEP regimens was previously assessed in the phase 2, 48-week, clinical trials; HPTN 069 and ACTG A5305. Efficacy was assessed in both men who have sex with men (MSM) and women who are at risk for HIV infection. Eligible participants received 1 of 4 MVC containing ARV regimens including; MVC alone (300 mg), MVC plus emtricitabine (300 mg; 200 mg, respectively), MVC plus tenofovir (300 mg; 300 mg, respectively) or tenofovir plus emtricitabine (300 mg; 200 mg, respectively) as a control arm. Among the 406 male participants, five acquired HIV infection (4 participants receiving MVC only, and 1 participant receiving MVC plus tenofovir). From the five participants who acquired HIV, 2 had undetectable drug concentrations at every visit, 2 had low concentrations at seroconversion and 1 participant had variable concentrations. Among the 188 female participants in the trial, none acquired HIV infection. MVC containing PrEP regimens were found to be safe and well tolerated compared with tenofovir/emtricitabine regimens in US men and women [32,33]

Here, we describe the use of an emulsion-templated freeze-drying (ETFD) technique [34] in the development of a MVC solid drug nanodispersion to investigate the potential of the formulation as a LAI. The standard MVC adult oral dose is 300 mg twice-daily, 600 mg twice-daily for patients receiving a CYP3A inducer (in the absence of a potent CYP3A inhibitor) and 150 mg twice-daily for patients receiving a CYP3A inhibitor [35]. In addition to being a CYP3A substrate, MVC is a P-glycoprotein (P-gp) substrate which reduces effective oral absorption [36]. Once absorbed, MVC is also a substrate for hepatic OATP1B1, which greatly facilitates its clearance from the systemic circulation [37]. It is estimated that over 60% of the absorbed drug is metabolised at first-pass, primarily by CYP3A, resulting in an estimated oral bioavailability of approximately 33% [38]. The extensive metabolism of MVC following oral administration and the need for dose adjustment make the development of an alternative dosing strategy particularly appealing. In this exploratory study we assessed the potential of a MVC nanodispersion as a LAI for use as PrEP using both *in vitro* release rate and *in vivo* pharmacokinetic approaches.

Experimental section

Materials

Dimethyl sulfoxide (DMSO), HEPES, bovine serum albumin (BSA), phosphate buffered saline (PBS), Hanks' balanced salt solution (HBSS), γ -globulin from bovine blood, dichloromethane, polyvinyl alcohol (PVA) and sodium 1,4-bis(2-ethylhexoxy)-1,4-dioxobutane-2-sulfonate (AOT) were all purchased from Sigma-Aldrich (UK). All other chemicals and reagents were purchased from Sigma-Aldrich (UK) and used as received, unless stated otherwise. Maraviroc was kindly gifted by ViiV Healthcare (UK) and [^3H]-maraviroc was purchased from Moravsek (US). Liquid scintillation fluid was purchased from Meridian biotechnologies (UK). Rapid Equilibrium Dialysis (RED) plates and inserts with a 8 kDa MWCO were purchased from Thermo Fisher Scientific (UK).

SDN MVC production and characterisation

MVC SDNs were prepared as described elsewhere in this issue [39]. Aqueous stock solutions of PVA and AOT were prepared at 22.5 mg ml, Maraviroc was prepared at 70 mg ml in dichloromethane. 70 wt% MVC loaded solid drug nanoparticles (SDN) stabilised with PVA and AOT ($^{\text{MVC}}\text{SDN}_{\text{PVA/AOT}}$) was prepared as followed: Solutions were prepared at a 4:1 water:oil mix, with 90 μl polymer (PVA), 45 μl surfactant (AOT) and 265 μl water added to 100 μl Maraviroc in DCM. The resulting mixture was emulsified with a Covaris S2x for 30 seconds with a duty cycle of 20, intensity of 10 and 500 cycles/burst in frequency sweeping mode, after which samples were immediately cryogenically frozen. Samples were then lyophilised using a Virtis benchtop K freeze dryers for 48 hours, and then sealed until analysis. Immediately prior to analysis, samples were dispersed in a volume of water to give 1 mg/ml concentration with respect to drug concentration. The z-average diameter (nm) of the SDNs was measured using dynamic light scattering (Malvern Zetasizer Nano ZS) using automatic measurement optimisation and Malvern Zetasizer software version 7.11 for data analysis.

Evaluation of MVC release rates using Rapid Equilibrium Dialysis (RED)

The rate of MVCs release from the SDN preparation was assessed across a size selective (8 kDa MWCO) membrane using RED plates and inserts (Thermo Fisher Scientific). Either Transport Buffer (TB) consisting of; Hanks balanced salt solution, 25 mM HEPES and 0.1% Bovine Serum Albumin (BSA), pH 7.4 or Simulated Interstitial Fluid (SIF) consisting of; dH_2O , 3.5% BSA and 0.2% γ -globulin, pH 7.4, were spiked with either DMSO dissolved MVC (<5% DMSO) or $^{\text{MVC}}\text{SDN}_{\text{PVA/AOT}}$. A total of 1 mg [^3H]-MVC (2 μCi mg) was added to the donor compartments for both preparations in 0.2 ml dH_2O with an additional 0.3 ml of either TB or SIF added to the donor chambers. One-millilitre of either TB or SIF was subsequently added to the corresponding acceptor chambers. The RED plates were sealed using Parafilm to avoid

evaporation and placed on an orbital shaker (Heidolph Rotomax 120; 100 rpm, 6 h, 37°C). Acceptor contents were subsequently sampled (0.6 ml) at 0.5, 1, 2, 3, 4, 5 and 6 h and replaced with an equal volume of fresh pre-warmed (37°C) SIF or TB. Collected samples (0.1 ml) were placed into empty 5 ml scintillation vials before mixing with liquid scintillation fluid (4 ml). Radioactivity was determined as disintegrations per minute (DPM) using a Packard Tri-carb 3100TR liquid scintillation counter. Data were expressed as the amount of [³H]-MVC released and diffused across the size selective membrane as a first-order release rate constant calculated over the 6 h incubation.

***In vivo* analysis of ^{MVC}SDN_{PVA/AOT} as a LAI**

All animal work was conducted in accordance with the Animals (Scientific Procedures) Act 1986 (ASPA) implemented by the UK Home Office. The rodents were housed with environmental enrichment and a 12 h light/dark cycle at 21°C ±2°C. Free access to food and water was provided at all times. Following 7-days acclimatisation, adult male Wistar rats (280-330 g) (Charles River, UK) were dosed intramuscularly with 10 mg/Kg MVC at 20 µCi/mg, after skin disinfection, with either a conventional [³H]-MVC preparation (<5% DMSO) or a [³H]-^{MVC}SDN_{PVA/AOT} nanodispersion into the left hind leg (musculus biceps femoris) using a 25G needle. Subsequently, blood samples were collected (0.25 ml) post-dosing from the tail vein until [³H]-MVC activity levels fell below the limits of detection (2 ng/ml). At the terminal timepoint, the rats were sacrificed using cardiac puncture under terminal anaesthesia (isoflurane/oxygen), followed by immediate exsanguination of blood from the heart. Subsequently, an overdose of sodium pentobarbitone (Animalcare, UK) was administered using the same in situ puncture needle.

Quantification of radiolabelled plasma

Blood samples were collected in heparinised Eppendorf tubes and centrifuged at 3,000 rpm for 5 min. The plasma layer was collected and stored at -20°C prior to analysis. Subsequently, 0.1 ml of each plasma sample was transferred into scintillation vials before adding scintillation fluid (4 ml) (Meridian Biotechnologies, UK) and scintillation counting using a Packard Tri-carb 3100TR.

Statistical analysis

Statistical analysis was performed using GraphPad Prism v.7 (US). Data normality was assessed with the Shapiro-Wilk test using StatsDirect v.3 (UK). Data were found to be normally distributed and unpaired, two-tailed t-tests were applied. For all comparisons, differences were considered statistically significant at *, P<0.05. Results are expressed as means and associated standard deviations. The pharmacokinetic parameters; maximum

217 concentration (C_{\max}), the time to C_{\max} (T_{\max}), trough concentrations (C_{\min}) and the average
218 concentration (C_{avg}) were derived from the concentration-time profiles. The area under the
219 curve, (AUC_{0-4} ; $AUC_{0-\infty}$) and terminal half-life ($t_{1/2}$) were calculated using PKSolver [40].
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Results and discussion

SDN materials and characterisation

^{MVC}SDN_{PVA/AOT} was prepared using an emulsion-templated freeze-drying method (EFTD) and was selected as the formulation for this study from a 49 screen matrix of polymer and surfactants, as previously described [39]. The formulation gave fully water dispersible solid drug nanoparticles with a hydrodynamic diameter in the region of 750 nm as measured by DLS. [³H]-MVC was incorporated into the formulation by spiking the initial MVC stock solution with the radiolabelled MVC. Incorporation of the radiolabelled [³H]-MVC does not affect the physical properties of the ^{MVC}SDN_{PVA/AOT}.

In vitro MVC release

An understanding of a formulation's *in vitro* release rate characteristics can be used to predict the rate of antiretroviral release from an intramuscular depot. Such information could potentially be used to predict dosage requirements that provide effective pharmacokinetic exposure relative to antiretroviral potency [41]. The rate of [³H]-MVC release from the ^{MVC}SDN_{PVA/AOT} was assessed across a size selective membrane (8 kDa MWCO) using two relevant buffers and compared to an equivalent conventional preparation of [³H]-MVC (<5% DMSO). The first-order release rate constant results, outlined in Fig. 1, indicate a reduction in MVC release rate and subsequent diffusion across the size selective membrane when formulated as ^{MVC}SDN_{PVA/AOT} in both TB and SIF. Specifically, MVC release rate constant was shown to be 22.7% and 10% lower for ^{MVC}SDN_{PVA/AOT} compared to the release rate constant for the conventional preparation in TB and SIF, respectively. Interestingly, the overall rate of MVC release for both preparations was increased in SIF compared to TB which is possibly attributed to the higher protein content of the SIF buffer. Given the modified *in vitro* release rates, an *in vivo* assessment of [³H]-MVCs exposure following intramuscular injection was warranted.

In vivo LAI MVC study

A rat model was used to investigate the potential of ^{MVC}SDN_{PVA/AOT} as a long-acting formulation. MVC exposure was assessed following a single intramuscular injection of either the ^{MVC}SDN_{PVA/AOT} nanodispersion or a conventional MVC preparation (<5% DMSO). A dose of 10 mg/Kg [³H]-MVC was injected into the left hind leg of each rat and blood samples were collected until [³H]-MVC activity levels fell below the limits of detection. The results in Fig. 2. show both [³H]-MVC exposure over the initial 24 h (insert), encompassing the 'burst event' and exposure for the duration of the procedure, until [³H]-MVC plasma concentrations fell below the limits of quantification. The pharmacokinetic parameters outlined in Table 1. show

a comparable C_{\max} (72.96 vs 71.67 ng/ml), an increase in T_{\max} (time to achieve C_{\max} after dosing, 2.0 vs. 1.0 h), increased AUC_{0-24} (652.66 vs. 244.29 ng.h/ml), increased $AUC_{0-\infty}$ (1959.71 vs. 567.17 ng.h/ml) and increased terminal half-life ($t_{1/2}$) (140.69 vs. 53.23 h) for the nanodispersion dosed rats. Following the initial rapid release of MVC, which led to the pronounced peak in plasma concentrations, the concentrations declined to 5.13% and 11.42% of the C_{\max} value within 24 h for the conventional and $^{MVC}SDN_{PVA/AOT}$ preparations, respectively. After 24 h the [3H]-MVC plasma concentrations remained comparatively stable, declining steadily so that [3H]-MVC was detectable for 3- and 10-days post-dosing for the conventional and nanodispersion preparations, respectively. It is interesting to note that comparable MVC concentrations were observed at 1-week post-dosing (C_{240}) for $^{MVC}SDN_{PVA/AOT}$ and 1-day post-dosing (C_{24}) for the conventional MVC preparation (2.08 ng/ml vs. 3.67 ng/ml). The terminal half-life ($t_{1/2}$) for orally dosed MVC is ~17 h compared to an observed ($t_{1/2}$) of 53.23 h and 140.69 h for the intramuscularly dosed conventional MVC preparation and $^{MVC}SDN_{PVA/AOT}$, respectively. Relatively low inter-individual variability was also noted for both treatment groups.

This exploratory study has identified a MVC nanodispersion with enhanced exposure compared to a conventional injected preparation. As outlined above, parenteral nanodispersions would appear to offer a simple and effective way of drug delivery and can provide unique benefits over current oral dosing strategies. However, the complex physiochemical properties and molecular mechanisms that allow for and influence protracted drug release from LAI nanodispersions are currently poorly understood [17]. If pharmacokinetic variability is driven by the rate of drug release from the injected depot then there are likely to be a number of depot specific physiological, anatomical and environmental factors that contribute to drug exposure, the potential significance of each of these has been reviewed recently [17]. An improved understanding of the mechanisms that permit extended drug release and protracted systemic drug exposure from LAI drug depots will ultimately help inform future nanoformulation designs and optimise release characteristics for particular diseases. Previous mechanistic studies into the tissue response to subcutaneous norethindrone implants (85% norethindrone, 15% cholesterol) may provide some insight into the mechanism that underpin drug release and exposure characteristics from LAI depots. In the study, microscopy was used to assess whether inflammatory responses played a role in drug absorption from the implants, in rats. It was noted that a dense fibrous biological compartment was formed around the implanted rods. The cellular tissue surrounding the rods was mainly composed of lipid laden macrophages which were contained within a fibrous envelope consisting of blood and lymphatic vessels. Increasing levels of norethindrone was observed in the formed tissue capsules, between 3 and 10.5 months post implantation. It was

suggested that the local inflammatory response played a substantial role in the processing of the implant drug delivery system [42].

Of particular interest for ARV therapy is the potential role of macrophages in enhancing drug distribution from the injected depot site. Macrophages have a critical role in HIV transmission, dissemination and are thought to act as reservoirs of the virus throughout infection [43,44]. Multimodal molecular imaging in rats has been used to assess the location of a LAI cabotegravir intramuscular depot and used to monitor volumetric and physiological changes at the depot site. Early rapid expansion of the cabotegravir depot volume was noted and associated with increased macrophage accumulation and subclinical oedema in and around the depot region, which was not identified in the vehicle control. Additionally, cabotegravir plasma concentrations were related to depot expansion within the first 4-days post administration [25]. Studies into the local disposition of the antipsychotic drug paliperidone palmitate, a solid drug particle preparation, identified the development of a subclinical but chronic granulomatous inflammatory reaction initiated by the presence of the solid material following intramuscular injection in rats. Macrophages were shown to be recruited to the formulation depot site and phagocytosed large fractions of the injected depot which influenced the rate of drug release. Microscopy also revealed the presence of particle loaded macrophages, with the highest density located adjacent to the depot site. Particle loaded macrophages were also observed in the local draining lymph nodes [22]. This is of interest to ARV therapy as lymph nodes are major sanctuary sites for HIV and ongoing viral replication occurs in lymph nodes even when virus is undetectable in circulating blood [4,45]. Inflammatory processes are known to evoke lymphangiogenesis [46] which may contribute to enhanced lymphatic drainage from the depot.

Further mechanistic studies were undertaken to investigate the effects of local macrophage infiltration and angiogenesis of the paliperidone palmitate prodrug depot. Paliperidone palmitate and paliperidone pharmacokinetics were assessed in rats following co-administration of the inhibitors liposomal clodronate and sunitinib. Clodronate was used to inhibit the recruitment of macrophages towards the depot injection site and subsequent sequestration of the paliperidone palmitate depot. Sunitinib is a potent vascular endothelial growth factor (VEGF) receptor antagonist and tyrosine kinase inhibitor and was used to inhibit the local neovascularization of the paliperidone palmitate depot. Co-administration of clodronate decreased the rate at which the granulomatous reaction formed and macrophage infiltration into the paliperidone palmitate depot was slowed. This was shown to slow the rate of prodrug dissolution and conversion to the active form, demonstrated by the delayed paliperidone T_{max} . Co-administration of sunitinib was shown to completely suppress the

granulomatous reaction and inhibited the neovascularization of the paliperidone palmitate depot. Co-incubation with sunitinib was shown to delay paliperidone T_{max} even further and reduced the C_{max} from 89.0 mg/ml to 41.7 ng/ml. This suggests macrophage infiltration and subsequent phagocytosis of the paliperidone palmitate depot actively contributed to paliperidone plasma exposure by promoting prodrug dissolution and conversion from paliperidone palmitate to paliperidone. It also highlights the role of angiogenesis in enhancing the absorptive capacity around the depot site [24].

The C_{max} of the standard 300 mg MVC twice-daily oral regimen, at steady state, is 724.9 ng/ml with a T_{max} and half-life of 3 h and 17 h, respectively [47]. The recommended minimum effective concentration for MVC therapy in HIV-1 infected adults and adolescents is between 25 and 50 ng/ml depending on regimen followed [48,49]. Although C_{avg} is an established parameter relating to orally-dosed MVC efficacy [47], it is unlikely to be an appropriate comparison for LAIs particularly for PrEP applications. The results highlighted here suggest up to 10-days MVC exposure following intramuscular injection in rats. Clearly, for an LAI MVC preparation to be effective in humans a MVC plasma concentration above 25 ng/ml would need to be attained and sustained for at least 7-days. Inference of $^{MVC}SDN_{PVA/AOT}$'s long-acting potential in humans is difficult as interspecies pharmacokinetic scaling is complex [50]. Difference in muscle structure/density and metabolic processes between species are likely to influence pharmacokinetics. Additionally, differences in the ratio of formulation injection volume to muscle volume, between species, may have a direct effect on drug exposure (e.g. a more substantial 'bust effect' may be anticipated with a higher injection volume to muscle volume due to increased muscle stretching caused by the newly formed depot).

Conclusions

Suboptimal adherence to daily oral antiretroviral therapy continues to hinder the efficacy of HIV treatment and PrEP. The development of alternative drug administration strategies such as LAIs that provide bi-monthly, monthly or even less frequent administration intervals are emerging and may mitigate some shortfalls of current oral regimens [17]. Patients frequently experience "pill fatigue" following prolonged oral daily dosing, and attitude surveys have consistently demonstrated enthusiasm for LAI in both HIV therapy and PrEP [51,52]. In this exploratory study the $^{MVC}SDN_{PVA/AOT}$ nanodispersion was developed and investigated for its potential as a LAI formulation. *In vitro* release rate assays revealed a reduced release rate constant for the nanodispersion compared to a conventional preparation of MVC. *In vivo* pharmacokinetic studies in rat demonstrated that MVC concentrations were detectable for up to 10-days, and cross-species differences in clearance may result in longer exposures in humans. Given the observed extended plasma exposure, further studies into MVC distribution

369 into biologically relevant tissues following intramuscular injection of the nanosuspension is
370 warranted.
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References

- [1] Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents
Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents
What's New in the Guidelines? Key Updates What to Start: Initial Combination
Regimens for the Antiretroviral-Naive Patient, (n.d.). <https://aidsinfo.nih.gov/guidelines>
(accessed October 12, 2017).
- [2] C. Trezza, S.L. Ford, W. Spreen, R. Pan, S. Piscitelli, Formulation and pharmacology
of long-acting cabotegravir, *Curr. Opin. HIV AIDS*. 10 (2015) 239–245.
doi:10.1097/COH.0000000000000168.
- [3] M.S. Cohen, Y.Q. Chen, M. Mccauley, T. Gamble, M.C. Hosseinipour, N. Kumarasamy,
J.G. Hakim, J. Kumwenda, B. Grinsztejn, J.H.S. Pilotto, S. V Godbole, S. Mehendale,
S. Chariyalertsak, B.R. Santos, K.H. Mayer, I.F. Hoffman, S.H. Eshleman, E. Piwowar-
Manning, L. Wang, J. Makhema, L.A. Mills, G. De Bruyn, I. Sanne, J. Eron, J. Gallant,
D. Havlir, S. Swindells, H. Ribaud, V. Elharrar, D. Burns, T.E. Taha, K. Nielsen-Saines,
D. Celentano, M. Essex, T.R. Fleming, Prevention of HIV-1 Infection with Early
Antiretroviral Therapy, *N Engl J Med*. 365(2011) 493–505.
doi:10.1056/NEJMoa1105243.
- [4] C. V. Fletcher, K. Staskus, S.W. Wietgreffe, M. Rothenberger, C. Reilly, J.G. Chipman,
G.J. Beilman, A. Khoruts, A. Thorkelson, T.E. Schmidt, J. Anderson, K. Perkey, M.
Stevenson, A.S. Perelson, D.C. Douek, A.T. Haase, T.W. Schacker, Persistent HIV-1
replication is associated with lower antiretroviral drug concentrations in lymphatic
tissues, *Proc. Natl. Acad. Sci.* 111 (2014) 2307–2312. doi:10.1073/pnas.1318249111.
- [5] R.J.Y. Ho, J. Yu, B. Li, J.C. Kraft, J.P. Freeling, J. Koehn, J. Shao, Systems Approach
to targeted and long-acting HIV/AIDS therapy, *Drug Deliv. Transl. Res.* 5 (2015) 531–
539. doi:10.1007/s13346-015-0254-y.
- [6] R.M. Grant, J.R. Lama, P.L. Anderson, V. McMahan, A.Y. Liu, L. Vargas, P. Goicochea,
M. Casapía, J.V. Guanira-Carranza, M.E. Ramirez-Cardich, O. Montoya-Herrera, T.
Fernández, V.G. Veloso, S.P. Buchbinder, S. Chariyalertsak, M. Schechter, L.-G.
Bekker, K.H. Mayer, G. Kallás, K.R. Amico, K. Mulligan, L.R. Bushman, R.J. Hance, C.
Ganoza, P. Defechereux, B. Postle, F. Wang, J.J. McConnell, J.-H. Zheng, J. Lee, J.F.
Rooney, H.S. Jaffe, A.I. Martinez, R. Ph, D.N. Burns, D. V Glidden, Preexposure
Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men, *N Engl J Med*.
363(2010). doi:10.1056/NEJMoa1011205.
- [7] WHO, WHO implementation tool for pre-exposure prophylaxis (PrEP) of HIV infection,
Hiv/Aids. (2017).
<http://www.who.int/hiv/pub/prep/prep-implementationtool/en/>
- [8] Truvada prescribing information,

- http://www.gilead.com/~media/Files/pdfs/medicines/hiv/truvada/truvada_pi.PDF
(accessed October 12, 2017).
- [9] C.W. Hendrix, Minireview Exploring Concentration Response in HIV Pre-Exposure Prophylaxis to Optimize Clinical Care and Trial Design, *Cell*. 155 (2013) 515–518. doi:10.1016/j.cell.2013.09.030.
- [10] O. Roberts, R.K.R. Rajoli, D.J. Back, A. Owen, K.M. Darin, C. V Fletcher, M. Lamorde, K.K. Scarsi, M. Siccardi, Physiologically based pharmacokinetic modelling prediction of the effects of dose adjustment in drug–drug interactions between levonorgestrel contraceptive implants and efavirenz-based ART. doi:10.1093/jac/dkx515.
- [11] JADELLE □ levonorgestrel implants.
https://www.accessdata.fda.gov/drugsatfda_docs/label/2002/20544se2-003_jadelle_lbl.pdf (accessed March 29, 2018).
- [12] M. Gunawardana, M. Remedios-Chan, C.S. Miller, R. Fanter, F. Yang, M.A. Marzinke, C.W. Hendrix, M. Beliveau, J.A. Moss, T.J. Smith, M.M. Baum, Pharmacokinetics of long-acting tenofovir alafenamide (GS-7340) subdermal implant for HIV prophylaxis, *Antimicrob. Agents Chemother.* 59 (2015) 3913–3919. doi:10.1128/AAC.00656-15.
- [13] G.J. Gatto, N. Girouard, R.M. Brand, L. Johnson, M.A. Marzinke, S. Rowshan, J. Engstrom, I. McGowan, Z. Demkovich, E. Luecke, A. Van Der Straten, Pharmacokinetics of tenofovir alafenamide by subcutaneous implant for HIV PrEP.
http://www.croiconference.org/sites/default/files/posters-2018/1430_Gatto_486.pdf
(accessed March 29, 2018).
- [14] N.M. Furiak, H. Ascher-Svanum, R.W. Klein, L.J. Smolen, A.H. Lawson, W. Montgomery, R.R. Conley, Cost-effectiveness of olanzapine long-acting injection in the treatment of patients with schizophrenia in the United States: a micro-simulation economic decision model, *Curr. Med. Res. Opin.* 27 (2011) 713–730. doi:10.1185/03007995.2011.554533.
- [15] D. Berardis, S. Marini, A. Carano, A. Lang, M. Cavuto, M. Piersanti, M. Fornaro, G. Perna, A. Valchera, M. Mazza, F. Iasevoli, G. Martinotti, M. Giannantonio, Efficacy and Safety of Long Acting Injectable Atypical Antipsychotics: A Review, *Curr. Clin. Pharmacol.* 8 (2013) 256–264. doi:10.2174/15748847113089990056.
- [16] J.A. Sierra-Ramírez, R. Lara-Ricalde, M. Lujan, N. Velázquez-Ramírez, M. Godínez-Victoria, I.A. Hernández-Munguía, A. Padilla, J. Garza-Flores, Comparative pharmacokinetics and pharmacodynamics after subcutaneous and intramuscular administration of medroxyprogesterone acetate (25 mg) and estradiol cypionate (5 mg), *Contraception*. 84 (2011) 565–570. doi:10.1016/j.contraception.2011.03.014.
- [17] A. Owen, S. Rannard, Strengths, weaknesses, opportunities and challenges for long acting injectable therapies: Insights for applications in HIV therapy, *Adv. Drug Deliv.*

- Rev. 103 (2016) 144–156. doi:10.1016/j.addr.2016.02.003.
- [18] P.E. Williams, H.M. Crauwels, E.D. Basstanie, Formulation and pharmacology of long-acting rilpivirine, *Curr. Opin. HIV AIDS*. 10 (2015) 233–238. doi:10.1097/COH.0000000000000164.
- [19] D.A. Margolis, J. Gonzalez-Garcia, H.J. Stellbrink, J.J. Eron, Y. Yazdanpanah, D. Podzamczar, T. Lutz, J.B. Angel, G.J. Richmond, B. Clotet, F. Gutierrez, L. Sloan, M.S. Clair, M. Murray, S.L. Ford, J. Mrus, P. Patel, H. Crauwels, S.K. Griffith, K.C. Sutton, D. Dorey, K.Y. Smith, P.E. Williams, W.R. Spreen, Long-acting intramuscular cabotegravir and rilpivirine in adults with HIV-1 infection (LATTE-2): 96-week results of a randomised, open-label, phase 2b, non-inferiority trial, *Lancet*. 390 (2017) 1499–1510. doi:10.1016/S0140-6736(17)31917-7.
- [20] B. Sillman, A.N. Bade, P.K. Dash, B. Bhargavan, T. Kocher, S. Mathews, H. Su, G.D. Kanmogne, L.Y. Poluektova, S. Gorantla, J. Mcmillan, N. Gautam, Y. Alnouti, B. Edagwa, H.E. Gendelman, Creation of a long-acting nanoformulated dolutegravir. doi:10.1038/s41467-018-02885-x.
- [21] R.J. Landovitz, R. Kofron, M. McCauley, The promise and pitfalls of long-acting injectable agents for HIV prevention., *Curr. Opin. HIV AIDS*. 11 (2016) 122–8. doi:10.1097/COH.0000000000000219.
- [22] N. Darville, M. van Heerden, A. Vynckier, M. De Meulder, P. Sterkens, P. Annaert, G. Van den Mooter, Intramuscular administration of paliperidone palmitate extended-release injectable microsuspension induces a subclinical inflammatory reaction modulating the pharmacokinetics in rats., *J. Pharm. Sci.* 103 (2014) 2072–87. doi:10.1002/jps.24014.
- [23] N. Darville, M. Van Heerden, T. Erkens, S. De Jonghe, A. Vynckier, M. De Meulder, A. Vermeulen, P. Sterkens, P. Annaert, G. Van Den Mooter, Modeling the Time Course of the Tissue Responses to Intramuscular Long-acting Paliperidone Palmitate Nano- / Microcrystals and Polystyrene Microspheres in the Rat, (2015). doi:10.1177/0192623315618291.
- [24] N. Darville, M. Van Heerden, D. Mariën, M. De Meulder, S. Rossenu, A. Vermeulen, A. Vynckier, S. De Jonghe, P. Sterkens, P. Annaert, G. Van Den Mooter, The effect of macrophage and angiogenesis inhibition on the drug release and absorption from an intramuscular sustained-release paliperidone palmitate suspension, *J. Control. Release*. 230 (2016) 95–108. doi:10.1016/j.jconrel.2016.03.041.
- [25] B.M. Jucker, H. Alsaid, M. Rambo, S.C. Lenhard, B. Hoang, F. Xie, M.R. Groseclose, S. Castellino, V. Damian, G. Bowers, M. Gupta, Multimodal imaging approach to examine biodistribution kinetics of Cabotegravir (GSK1265744) long acting parenteral formulation in rat, *J. Control. Release*. 268 (2017) 102–112.

doi:10.1016/j.jconrel.2017.10.017.

- [26] K.C. Brown, K.B. Patterson, S. a. Malone, N.J. Shaheen, H.M.A. Prince, J.B. Dumond, M.B. Spacek, P.E. Heidt, M.S. Cohen, A.D.M. Kashuba, Single and multiple dose pharmacokinetics of maraviroc in saliva, semen, and rectal tissue of healthy HIV-negative men, *J. Infect. Dis.* 203 (2011) 1484–1490. doi:10.1093/infdis/jir059.
- [27] J.B. Dumond, K.B. Patterson, A.L. Pecha, R.E. Werner, E. Andrews, B. Damle, R. Tressler, J. Worsley, A.D.M. Kashuba, Maraviroc Concentrates in the Cervicovaginal Fluid and Vaginal Tissue of HIV-Negative Women, *JAIDS J. Acquir. Immune Defic. Syndr.* 51 (2009) 546–553. doi:10.1097/QAI.0b013e3181ae69c5.
- [28] U.M. Parikh, J.W. Mellors, Should we fear resistance from tenofovir / emtricitabine preexposure prophylaxis ?, 11 (2016) 49–55. doi:10.1097/COH.0000000000000209.
- [29] A.E. Petroll, J.L. Walsh, J.L. Owczarzak, T.L. McAuliffe, L.M. Bogart, J.A. Kelly, PrEP Awareness, Familiarity, Comfort, and Prescribing Experience among US Primary Care Providers and HIV Specialists, *AIDS Behav.* 21 (2017) 1256–1267. doi:10.1007/s10461-016-1625-1.
- [30] C.M. Perry, Maraviroc, *Drugs.* 70 (2010) 1189–1213. doi:10.2165/11203940-000000000-00000.
- [31] J. Fox, J.M. Tiraboschi, C. Herrera, L. Else, D. Egan, L. Dickinson, A. Jackson, N. Olejniczak, D. Back, S. Khoo, R. Shattock, M. Boffito, Brief Report: Pharmacokinetic/Pharmacodynamic Investigation of Single-Dose Oral Maraviroc in the Context of HIV-1 Pre-exposure Prophylaxis, *JAIDS J. Acquir. Immune Defic. Syndr.* 73 (2016) 252–257. doi:10.1097/QAI.0000000000001108.
- [32] R.M. Gulick, T.J. Wilkin, Y.Q. Chen, R.J. Landovitz, K.R. Amico, A.M. Young, P. Richardson, M.A. Marzinke, C.W. Hendrix, S.H. Eshleman, I. McGowan, L.M. Cottle, A. Andrade, C. Marcus, K.L. Klingman, W. Chege, A.R. Rinehart, J.F. Rooney, P. Andrew, R.A. Salata, M. Magnus, J.E. Farley, A. Liu, I. Frank, K. Ho, J. Santana, J.D. Stekler, M. McCauley, K.H. Mayer, Phase 2 Study of the Safety and Tolerability of Maraviroc-Containing Regimens to Prevent HIV Infection in Men Who Have Sex With Men (HPTN 069/ACTG A5305), *J. Infect. Dis.* 215 (2017) 238–246. doi:10.1093/infdis/jiw525.
- [33] G. R.M., W. T.J., C. Y.Q., L. R.J., A. K.R., Y. A.M., R. P., M. M.A., H. C.W., E. S.H., M. I., C. L.M., A. A., M. C., K. K.L., C. W., R. A.R., R. J.F., A. P., S. R.A., S. M., M. Y.C., F. I., H. K., S. J., S. J.D., S. S., M. M., H. S., M. K.H., Safety and tolerability of maraviroc-containing regimens to prevent HIV infection in women, *Ann. Intern. Med.* 167 (2017) 384–393. doi:10.7326/M17-0520.
- [34] T.O. McDonald, M. Giardiello, P. Martin, M. Siccardi, N.J. Liptrott, D. Smith, P. Roberts, P. Curley, A. Schipani, S.H. Khoo, J. Long, A.J. Foster, S.P. Rannard, A. Owen, Antiretroviral Solid Drug Nanoparticles with Enhanced Oral Bioavailability: Production,

- Characterization, and In Vitro-In Vivo Correlation, *Adv. Healthc. Mater.* 3 (2014) 400–411. doi:10.1002/adhm.201300280.
- [35] CELSENTRI | ViiV Healthcare Exchange.
<https://uk.viivexchange.com/our-medicines/celsentri/> (accessed October 17, 2017).
- [36] D.K. Walker, S.J. Bowers, R.J. Mitchell, M.J. Potchoiba, C.M. Schroeder, H.F. Small, Preclinical assessment of the distribution of maraviroc to potential human immunodeficiency virus (HIV) sanctuary sites in the central nervous system (CNS) and gut-associated lymphoid tissue (GALT)., *Xenobiotica*. 38 (2008) 1330–9. doi:10.1080/00498250802447409.
- [37] M. Siccardi, A. D'Avolio, S. Nozza, M. Simiele, L. Baietto, F.R. Stefani, D. Moss, W.S. Kwan, A. Castagna, A. Lazzarin, A. Calcagno, S. Bonora, D. Back, G. Di Perri, A. Owen, Maraviroc is a substrate for OATP1B1 in vitro and maraviroc plasma concentrations are influenced by SLCO1B1 521 T>C polymorphism, *Pharmacogenet. Genomics*. 20 (2010) 759–765. doi:10.1097/FPC.0b013e3283402efb.
- [38] S. Abel, D. Russell, L.A. Whitlock, C.E. Ridgway, A.N.R. Nedderman, D.K. Walker, Assessment of the absorption, metabolism and absolute bioavailability of maraviroc in healthy male subjects., *Br. J. Clin. Pharmacol.* 65 Suppl 1 (2008) 60–7. doi:10.1111/j.1365-2125.2008.03137.x.
- [39] A.C. Savage, L.M. Tatham, M. Siccardi, T. Scott, S.P. Rannard, A. Owen, Improving Maraviroc Oral Bioavailability by Nanoformulation, *Eur. J. Pharm. Biopharm.* (n.d.).
- [40] Y. Zhang, M. Huo, J. Zhou, S. Xie, PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel, (2010). doi:10.1016/j.cmpb.2010.01.007.
- [41] R.K.R. Rajoli, D.J. Back, S. Rannard, C.L. Freel Meyers, C. Flexner, A. Owen, M. Siccardi, Physiologically Based Pharmacokinetic Modelling to Inform Development of Intramuscular Long-Acting Nanoformulations for HIV, *Clin. Pharmacokinet.* 54 (2015) 639–650. doi:10.1007/s40262-014-0227-1.
- [42] F.D. Anderson, D.F. Archer, S.M. Harman, R.J. Leonard, W.H. Wilborn, Tissue response to bioerodible, subcutaneous drug implants: a possible determinant of drug absorption kinetics., *Pharm. Res.* 10 (1993) 369–80.
- [43] S.R. DiNapoli, V.M. Hirsch, J.M. Brenchley, Macrophages in Progressive Human Immunodeficiency Virus / Simian Immunodeficiency Virus Infections, *J Virol.* 90 (2016) 7596–7606. doi:10.1128/JVI.00672-16.Editor.
- [44] J.H. Campbell, A.C. Hearps, G.E. Martin, K.C. Williams, S.M. Crowe, The importance of monocytes and macrophages in HIV pathogenesis, treatment, and cure, *Aids*. 28 (2014) 2175–2187. doi:10.1097/QAD.0000000000000408.
- [45] J.N. Blankson, D. Persaud, R.F. Siliciano, P.R. In, P.A.R. Ecyling, The challenge of

- viral reservoirs in HIV-1 infection, *Annu. Rev. Med.* 53 (2011) 9–10. doi:10.1093/ajae/aau087.
- [46] M.J. Flister, A. Wilber, K.L. Hall, C. Iwata, K. Miyazono, R.E. Nisato, M.S. Pepper, D.C. Zawieja, S. Ran, Inflammation induces lymphangiogenesis through up-regulation of VEGFR-3 mediated by NF- κ B and Prox1, 115 (2010) 418–429.
- [47] S. Abel, D.J. Back, M. Vourvahis, Maraviroc: pharmacokinetics and drug interactions., *Antivir. Ther.* 14 (2009) 607–18.
- [48] S.M. Woollard, G.D. Kanmogne, Maraviroc a review of its use in HIV infection and beyond, *Drug Des. Devel. Ther.* 9 (2015) 5447–5468. doi:10.2147/DDDT.S90580.
- [49] J. Sierra-Madero, G. Di Perri, R. Wood, M. Saag, I. Frank, C. Craig, R. Burnside, J. McCracken, D. Pontani, J. Goodrich, J. Heera, H. Mayer, Efficacy and safety of maraviroc versus efavirenz, both with zidovudine/lamivudine: 96-week results from the MERIT study, *HIV Clin Trials.* 11 (2010) 125–132. doi:10.1310/hct1103-125.
- [50] J.H. Lin, ANTHONY Y. H. LU, Applications and limitations of interspecies scaling and in vitro extrapolation in pharmacokinetics. <http://www.dmd.org> (accessed September 21, 2017).
- [51] J.T. Parsons, H.J. Rendina, T.H.F. Whitfield, C. Grov, Familiarity with and Preferences for Oral and Long-Acting Injectable HIV Pre-exposure Prophylaxis (PrEP) in a National Sample of Gay and Bisexual Men in the U.S., *AIDS Behav.* 20 (2016) 1390–1399. doi:10.1007/s10461-016-1370-5.
- [52] J. Williams, H.R. Sayles. J.L. Meza, P. Sayre, U. Sandkovsky, H.E. Gendelman, C. Flexner, S. Swindells. Long-acting parenteral nanoformulated antiretroviral therapy : interest and attitudes of HIV-infected patients, *Nanomed.* 8 (2013) 1807–1813. doi: 10.2217/nnm.12.214.

Tables & Figures

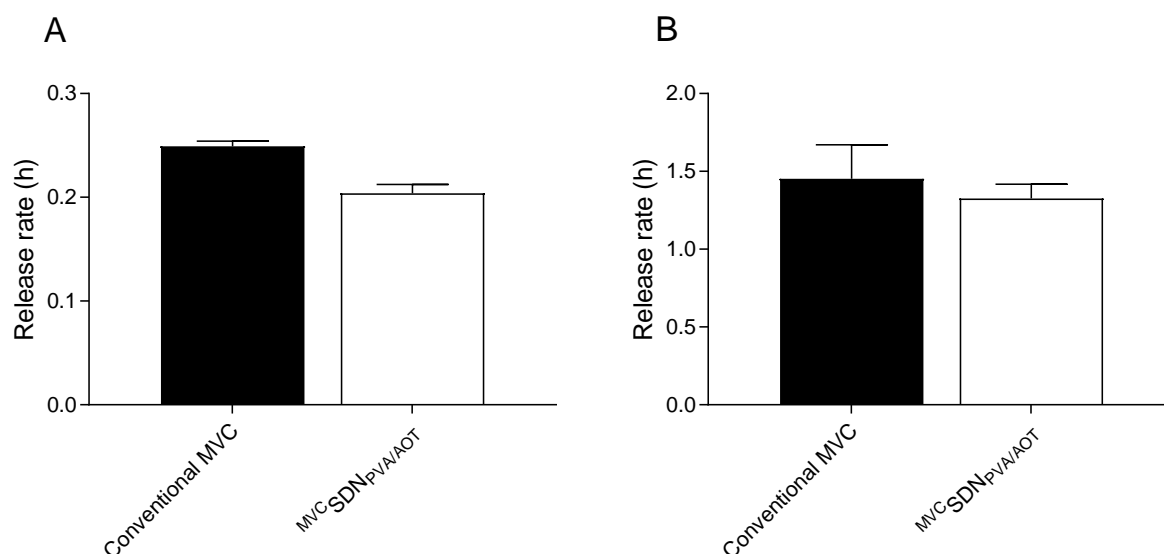


Figure 1. $[^3\text{H}]$ -MVC release rate across a size selective membrane for both a conventional $[^3\text{H}]$ -MVC preparation (<5% DMSO) or the nanodispersion $^{\text{MVC}}\text{SDN}_{\text{PVA/AOT}}$ using either transport buffer (A) or simulated interstitial fluid (B) as both donor and acceptor media in a RED assay. The average release rate constant was calculated over 6 h for each preparation and the error bars give the standard deviations of the mean from three replicates.

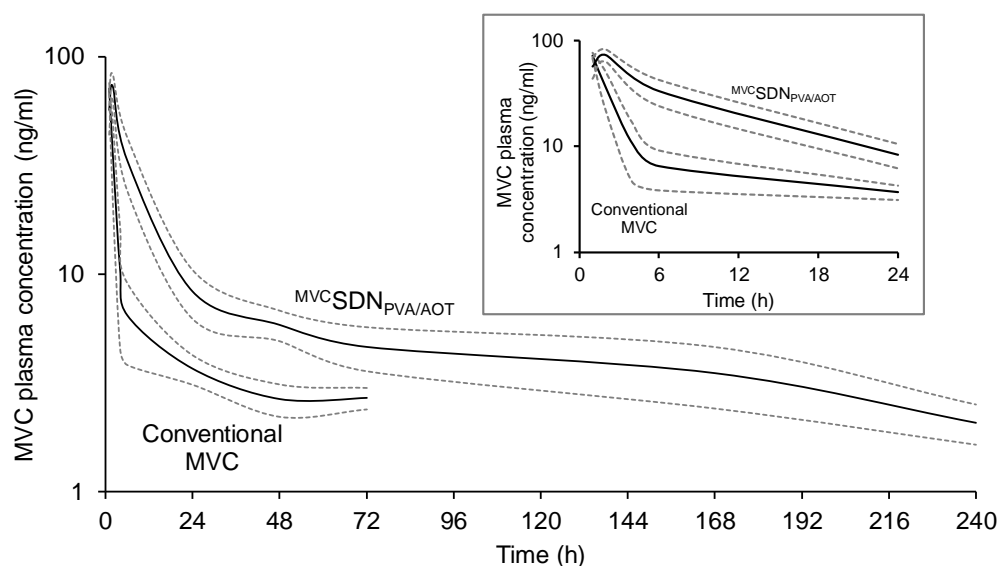


Figure 2. MVC exposure in adult male Wistar rats following a single intramuscular injection of $[^3\text{H}]$ -MVC (10 mg/Kg, 20 μCi mg $[^3\text{H}]$ -activity) in the biceps femoris either as a conventional preparation (<5% DMSO) or as the nanodispersion $^{\text{MVC}}\text{SDN}_{\text{PVA/AOT}}$. Data is expressed as plasma concentrations of $[^3\text{H}]$ -MVC over the initial 24 h (insert) or until plasma concentrations fell below the limits of detection (<2 ng ml). The fragmented lines give the standard deviations of the mean for three rats in each group.

Table 1. The pharmacokinetic parameters of MVC following intramuscular injection of [³H]-MVC (10 mg Kg, 20 µCi mg [³H]-activity) in the biceps femoris either as a conventional preparation (<5% DMSO) or as the nanodispersion ^{MVC}SDN_{PVA/AOT}. Parameters were calculated from the exposure curves outlined in Fig. 2.

Pharmacokinetic parameter	Conventional MVC	^{MVC} SDN _{PVA/AOT}
C _{max} (ng/ml)	71.67	72.96
AUC _{t-∞} (ng.h/ml)	567.17	1959.71
AUC ₀₋₂₄ (ng.h/ml)	244.29	652.66
Terminal half-life t _{1/2} (h)	53.23	140.69
T _{max} (h)	1	2
C ₂₄ (ng/ml)	3.67	8.33
C ₄₈ (ng/ml)	2.69	5.85
C ₇₂ (ng/ml)	2.66	4.64
C ₁₆₈ (ng/ml)	-*	3.51
C ₂₄₀ (ng/ml)	-*	2.08

*Below limits of detection

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